

REMARKS

The specification has been amended to comply with the Sequence Listing requirements of 37 CFR §§1.821 *et seq.* Specifically, page 11 of the specification was amended such that SEQ ID NO:3 and SEQ ID NO:4, set forth in Fig. 2, and SEQ ID NO:27 and SEQ ID NO:28, set forth in Fig. 10c, are now referenced.

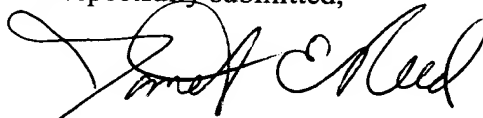
Additionally, this amendment directs replacement of the originally filed paper copy of the Sequence Listing with the paper copy of the amended Sequence Listing submitted herewith. The Sequence Listing was amended to include SEQ ID NOS: 19-28, which were set forth in the specification and drawings as filed, but which had not been included in the originally filed Sequence Listing. Hence, no new matter has been added by this amendment.

A computer-readable form of the amended Sequence Listing is also submitted herewith. Applicants' undersigned attorney verifies that the sequences set forth in the computer-readable form of the Sequence Listing are the same as those set forth in the paper copy of the Sequence Listing.

Applicants submit that the present application is in full compliance with all requirements of 37 CFR §§1.821 *et seq.* and is now in condition for examination on its merits.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Respectfully submitted,



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COPY OF PAPERS
ORIGINALLY FILED PATENT

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sequence Listing as amended:

SEQUENCE LISTING

<110> Tracy, Steven M.
Chapman, Nora M.
Kolbeck, Peter
Malone, James

<120> Cocksackievirus Vectors And Their Use In Prevention And Treatment Of
Disease

<130> UNMC0027 [UNMC 63116]

<140> 09/817,748

<141> 2001-03-27

<150> 09/403,672

<151> 2000-03-27

<150> PCT/US98/04291

<151> 1998-03-05

<150> 08/812,121

<151> 1997-03-05

<160> 28 [18]

<170> PatentIn version 3.1 [FastSEQ for Windows Version 3.0]

<210> 1

<211> 85

<212> DNA

<213> Cocksackievirus

<400> 1

atcactacaa tgacaaatac gggcgcatth ggacaacaat caaggggcag cgtatgtggg 60
gaactacagg gtaatgggtc tcaac 85

<210> 2

<211> 80

<212> DNA

<213> Cocksackievirus

<400> 2

tactcgatca ctacaatgac aaatacgggc gcatttggac aacaatcagg ggcagcgtat 60
gtggggaact acagggtagt 80

<210> 3

<211> 34

<212> PRT

<213> Cocksackievirus

<400> 3

Ser Gly Val Thr Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr
1 5 10 15

Gly Ala Phe Gly Gln Gln Ser Gly Ala Val Thr Leu Glu Met Pro Gly
20 25 30

Ser Ala

<210> 4

<211> 25

<212> PRT

<213> Cocksackievirus

<400> 4

Met Lys Ser Asn Ser Ile Thr Thr Met Thr Asn Thr Gly Ala Phe Gly
1 5 10 15

Gln Gln Ser Gly Ala Val Tyr Val Gly
20 25

<210> 5

<211> 27

<212> DNA

<213> Cocksackievirus

<400> 5

atgggaaatt cgagctcgat gcctggc

27

<210> 6

<211> 28

<212> DNA

<213> Cxsackievirus

<400> 6
atgaaaagcg catgcgggtt ttcaaggt

28

<210> 7

<211> 74

<212> DNA

<213> Cxsackievirus

<400> 7
actactaggc aaagcatcac tacaatgaca aatacgggcg catttggaca acaatcaggg
cagtctcgga tcca

60

74

<210> 8

<211> 62

<212> DNA

<213> Cxsackievirus

<400> 8
gaattctgca gatcaattac caccatgacc aacacggggc gcatttggac aatcaggggc
ag

60

62

<210> 9

<211> 5

<212> PRT

<213> Cxsackievirus

<400> 9

Asn Thr Gly Ala Phe

1

5

<210> 10

<211> 11

<212> PRT

<213> Cocksackievirus

<400> 10

Tyr Arg Val Met Gly Leu Asn Tyr Ser Ile Thr
1 5 10

<210> 11

<211> 58

<212> PRT

<213> Cocksackievirus

<400> 11

Ser Gly Val Thr Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr
1 5 10 15

Gly Ala Phe Gly Gln Gln Ser Gly Ala Val Thr Leu Glu Asp Pro Arg
20 25 30

Val Pro Ser Ser Asn Ser Ile Thr Thr Met Thr Asn Thr Gly Ala Phe
35 40 45

Gly Gln Gln Ser Gly Ala Val Tyr Val Gly
50 55

<210> 12

<211> 59

<212> PRT

<213> Cocksackievirus

<400> 12

Ser Gly Val Thr Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr
 1 5 10 15

Gly Ala Phe Gly Gln Gln Ser Gly Ala Val Thr Leu Glu Met Pro Gly
 20 25 30

Ser Ala Met Lys Ser Asn Ser Ile Thr Thr Met Thr Asn Thr Gly Ala
 35 40 45

Phe Gly Gln Gln Ser Gly Ala Val Tyr Val Gly
 50 55

<210> 13

<211> 69

<212> DNA

<213> Cocksackievirus

<400> 13

atgggaaatt cgagctccgt acccggggat cctctagagt cgacctgcag gcatgcgggt 60

tttcaagga 69

<210> 14

<211> 55

<212> DNA

<213> Cocksackievirus

<400> 14

atgggaaatt cgagctcgat gcctggcatg aaaagcgcat gcgggttttc aaggt 55

<210> 15

<211> 23

<212> PRT

<213> Cocksackievirus

<400> 15

Met Gly Asn Ser Ser Ser Val Pro Gly Asp Pro Leu Glu Ser Thr Cys
1 5 10 15

Arg His Ala Gly Phe Gln Gly
20

<210> 16

<211> 18

<212> PRT

<213> Cocksackievirus

<400> 16

Met Gly Asn Ser Ser Ser Met Pro Gly Met Lys Ser His Ala Gly Phe
1 5 10 15

Gln Gly

<210> 17

<211> 138

<212> DNA

<213> Cocksackievirus

<400> 17

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cgagtctcgg atccagaatt ctgcagatca attaccacca tgaccaaacac cggggcgcat 120

ttggacaatc aggggcag

138

<210> 18

<211> 46

<212> PRT

<213> Cocksackievirus

<400> 18

Thr Thr Arg Gln Ser Ile Thr Thr Met Thr Asn Thr Gly Ala Phe Gly
 1 5 10 15

Gln Gln Ser Gly Ala Val Ser Asp Pro Glu Phe Cys Arg Cys Ile Thr
 20 25 30

Thr Met Thr Asn Thr Gly Ala Phe Gly Gln Ser Gly Ala Val
 35 40 45

<210> 19

<211> 124

<212> DNA

<213> Cocksackievirus

<400> 19

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taggatatcc tgcaggatta caactatgac taacaccggg gctttcggtc agcagagtgg 120

ggca 124

<210> 20

<211> 41

<212> PRT

<213> Cocksackievirus

<400> 20

Ile	Thr	Thr	Met	Thr	Asn	Thr	Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
1				5					10					15	

Val	Ser	Asp	Pro	Arg	Ile	Ser	Cys	Arg	Ile	Thr	Thr	Met	Thr	Asn	Thr
		20						25					30		

Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
	35						40	

<210> 21

<211> 268

<212> DNA

<213> Cocksackievirus

<400> 21

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ggatgaaaaa ggggtgcctc ttccaaaggt tgacttgcaa ttcttctcaa atactacctc	120
tttgaacgac cggcaaggca atgctactaa accaaaagtg gttttgtaca gtgaagatgt	180
aaatatggaa accccagaca cacatctgtc tgactgcagg attacaacta tgactaacac	240
cggggcctttc ggtcagcaga gtggggca	268

<210> 22

<211> 89

<212> PRT

<213> Cocksackievirus

<400> 22

Ile	Thr	Thr	Met	Thr	Asn	Thr	Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
1				5					10					15	

PATENT

-15-

<213> Coxsackievirus

<400> 25

ctagactctg ccaatacga

20

<210> 26

<211> 21

<212> DNA

<213> Coxsackievirus

<400> 26

gtgctcacta agaggtctct g

21

<210> 27

<211> 49

<212> DNA

<213> Coxsackievirus

<400> 27

catcacaact atgactaaca ccggggcttt cggtcagcag agtggggca

49

<210> 28

<211> 16

<212> PRT

<213> Coxsackievirus

<400> 28

Ile	Thr	Thr	Met	Thr	Asn	Thr	Gly	Ala	Phe	Gly	Gln	Gln	Ser	Gly	Ala
1			5				10						15		

Amended paragraph beginning at page 11, line 25:

Figure 2 shows the amino acid sequence of the PLS-CVB3 genome (SEQ ID NO:11) and the mIL-10-CVB3 (SEQ ID NO:12) at the site of the protease 2A cleavage (in SEQ ID NO:12, the sequence to the left of A...M is SEQ ID NO:3 and the sequence to the right of A...M is SEQ ID NO:4). In this construct, the cloning procedure has been modified to include a polylinker site (PLS) to facilitate the use of the CVB3 as a generic cloning and expression vehicle. Further modifications include non-direct repeat genetic sequences to code for the protease P2-A cleavage site in the nascent polyprotein. The amino acids donated by the PLS are underlined, while the amino acids which form the 2A cleavage recognition signal are double underlined. The sequence of the mIL-10 insertion is shown in bold.

Amended paragraph beginning at page 14, line 27:

Figure 10 shows PCR and sequence analysis of CVB3-PL2-Ad2L1. pCVB3-PL2-Ad2L1 was transfected into HeLa cells, and the resultant progeny virus (CVB3-PL2-Ad2L1, pass 1) was subsequently serially passaged in HeLa cell cultures (passes 2 to 10). Viral RNA was isolated from virus stocks at each passage, and the presence of the inserted Ad2 sequence was analyzed by PCR using primers flanking the insertion site in the CVB3 genome. Fig. 10 a: amplimers were separated by agarose gel electrophoresis. CVB3-PL2-Ad2L1, RT-PCR amplimer using

chimeric viral RNA as the template; pCVB3-PL2-Ad2L1, PCR amplimer using the chimeric plasmid DNA as the template; CVB3/0, RT-PCR amplimer using the parental CVB3/0 RNA as template; neg., RT-PCR using RNA as template from uninfected HeLa cells; Marker, 100-bp DNA ladder. Fig. 10b and 10c: the sequence of the Ad2 insert-containing 446-bp amplimer (CVB3-PL2-Ad2L1) (Fig. 10b; nucleic acid sequence is SEQ ID NO:21; amino acid sequence is SEQ ID NO:22) and the sequence of the 225-bp Ad2 fragment-deleted amplimer (CVB3-PL2-Ad2L1del) (Fig. 10c; nucleotide sequence is SEQ ID NO:27 [comprises bases 1-49 of SEQ ID NO:21]; amino acid sequence is SEQ ID NO:28 [comprises residues 1-16 of SEQ ID NO:22]) were obtained after isolation of the DNA fragments from agarose gels. Sequence analysis was performed with the same primers as for the RT-PCR analysis. Numbering is based on the CVB3/0 genome (Genbank accession no. M88483).